(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2002 (21.11.2002)

PCT

(10) International Publication Number
WO 02/092161 A1

(51) International Patent Classification[7]: A61M 37/00, G01N 33/48

(21) International Application Number: PCT/US02/14253

(22) International Filing Date: 6 May 2002 (06.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/852,876 10 May 2001 (10.05.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 02/092161 A1

(54) Title: MINIATURIZED PARTICLE ANALYZER

(57) Abstract: A particle analyzer containing a device (12) for acquiring particles of cellular material from a living body, a device (42) for labeling the particles of cellular material to produce labeled particles, a device (82) for analyzing the labeled particles to produce analyzed and labeled particles, a device (118) for sorting the analyzed and labeled particles to produce labeled and sorted particles, and a device (120) for maintaining a portion of the analyzed and labeled particles in a viable state.

# MINIATURIZED PARTICLE ANALYZER

## Technical Field

A miniature particle analyzer, adapted to both detect and treat mammalian cells.

## Background Art

Flow cytometers are well known to those skilled in the art. By way of illustration and not limitation, one may refer to, e.g., United States patents 6,198,110, 6,197,744, 6,197,593, 6,197,540, 6,197,539, and the like. By way of further illustration, United States patent 6,097,485 discloses a miniature flow cytometer adapted to measure laser-induced fluorescence.

Most of the prior art flow cytometers are relatively large and unwieldy and, thus, cannot readily accompany a patient in his everyday endeavors. Furthermore, most prior art flow cytometers are not adapted to acquire and analyze cellular material directly from the patient; the particles analyzed with these prior art machines must be extracted from the body and prepared in vitro in an exogenous fluid medium.

It is an object of this invention to provide a particle analyzer that is portable, that can be affixed to the body of a patient, that can directly acquire and analyze cellular material, and that can treat cellular material.

It is another object of this invention to provide a particle analyzer that can be used therapeutically as well as diagnostically.

## Disclosure of the invention

In accordance with this invention, there is provided a particle analzyer which comprises means for acquiring particles of cellular material from a living body, a means for labeling said particles of cellular material within a bodily fluid with a label to produce labeled particles, means for analyzing the labeled particles, and means for sorting the labeled particles.

## Brief description of the drawings

The invention will be described by reference to the specification and to the following drawings, in which like numerals refer to like elements, and in which:

Figure 1 is a flow diagram of one preferred process of the invention;

Figure 2 is a schematic of one preferred assembly of the invention for acquiring, wherein the assembly is comprised of a particle analyzer;

Figures 3A, 3B, and 3C schematically illustrate the actions of the pump of the assembly depicted in Figure 2;

Figure 4 is a schematic diagram of one preferred means for preparing a bodily fluid for analysis;

Figure 5 is a schematic of the detection/treatment system of the particle analyzer assembly;

Figure 6 is a schematic of the particle analyzer assembly in relation to the location of bodily fluids;

Figure 7 is a schematic of one preferred means for maintaining a viable bodily fluid;

Figure 8 is a schematic of a particle analyzer disposed within a living body; and

Figure 9 is a schematic of a particle analyzer disposed outside of a living body;

Best Mode for Carrying Out the Invention

Process Steps

Figure 1 is a flow diagram of one preferred process 10 for analyzing, treating, and maintaining certain bodily fluids.

Acquiring

In step 12 of the process, the bodily fluids are acquired.

One may use any conventional means for acquiring the body fluids. The body fluids which are typically acquired include, e.g., blood, lymph, spinal fluid, semen, fat cells, stem cells, bone marrow, and the like.

In one embodiment, the body fluids are acquired by means of the sampling system described in United States patent 6,159,164, the entire disclosure of which is hereby incorporated by reference into this specification. The system of this patent samples a body fluid through a tube attached to a patient's body; and the system is operable by a user having a hand, including a palm, a thumb, and at least a first finger and a second finger. The system comprises a fluid sampling site connected to the tube; means for receiving the tube; means for forming a chamber; means for selectively increasing the size of the chamber to a maximum volume and for decreasing the size of the chamber to a minimum volume, the means for increasing and decreasing the size of the chamber being operable by moving the first and second fingers or the thumb in a flexion movement toward the palm to achieve the maximum volume of the chamber, the means for increasing and decreasing the size of the chamber also being operable by moving the first and second fingers or the thumb in a flexion movement toward the palm to achieve the minimum volume of the chamber such that the same motion of the user's first and second fingers can selectively accomplish the maximum volume to aspirate fluid from the patient's

body to the fluid sampling site or accomplish the minimum volume to expel the fluid into the patient's body.

Figures 2 and 3 indicate another acquiring assembly that may be used. Figure 2 outlines the pump's bodily location and Figure 3 details the pumping action. Referring to Figures 2 and 3, a patient has disposed within her body, beneath her diaphragm 16, a pump 18 that is actuated by the movement of diaphragm 16 in the direction of arrows 19 and 20.

Referring to Figure 3, the pump 18 has a deformable and elastic casing 22. When casing 22 is compressed between diaphragm 16 and abdominal wall 24, its interior volume will decrease, and fluid disposed within pump 18 will be discharged through line 26 to particle analyzer/flow cytometer 44.

The pump 18 comprises one-way flow valve 30, which allows fluid flow only in the direction of arrow 32; and it also comprises one-way flow valve 34, which only allows flow in the direction of arrow 36. Thus, when casing 22 is compressed, fluid only may flow through line 26; when the compressed casing 22 is allowed to expand to its original shape (when the diaphragm 16 relaxes), the fluid may flow only through line 38.

Although the pump 18 is shown disposed beneath the patient's diaphragm 16, it will be apparent that such pump 18 may be disposed beneath or nearby other parts of a body which expand and contract. Thus, by way of illustration and not limitation, the pump 18 may be positioned between lung and ribcage, between muscle and bone, between a heart and a sternum, and the like.

Referring again to Figure 2, it will be apparent that, every time the diaphragm 16 expands and thereafter contracts, fluid will be withdrawn from blood vessel 40 via line 38 into pump 18; and the fluid within such pump 18 will be fed to particle analyzer 44 via line 26 upon the next expansion of the diaphragm 16. This is one preferred means of acquiring the blood in blood vessel 40, and it operates continuously with the movement of diaphragm 16.

Figures 3A, 3B, and 3C illustrate the operation of pump 18 in its intake phase (Figure 3A), its expulsion phase (Figure 3B), and its subsequent intake phase (Figure 3C). The pump 18 is compressed when the diaphragm 16 moves in the direction of arrow 20; and it is allowed to return to its non-compressed state when the diaphragm 16 moves in the direction of arrow 19. In another embodiment, not shown, the pump 18 is replaced by a piezoelectric assembly (not shown) that, upon pressure being applied to it, produces a difference of potential sufficient to actuate a pump to which it is electrically connected.

Sample Acquisition

Referring again to Figure 1, in step 42 of the process, the bodily fluid which has been acquired is then prepared for analysis. One may use any method for enumerating and distinguishing between fluid cell populations in a bodily sample. Thus, by way of illustration and not limitation, one may use the method described in United States patent 6,197,593, the entire disclosure of which is hereby incorporated by reference into this specification.

In the first step of the process of U.S. 6,197,593, a biological sample is contacted with two or more blood cell populations with a selective nucleic acid specific blocking agent to form a sample mixture. The sample mixture is then contacted with a cell membrane permeant, red-excited dye without significantly disrupting cellular integrity of the cells to form a dyed sample mixture. The dyed sample mixture is excited with light in a single red wavelength; and, thereafter, fluorescence emitted from different cell populations in the dyed sample mixture is measured, wherein the fluorescence emitted from one blood cell population is distinguishable from the fluorescence emitted from another blood cell population.

Referring to Figure 4, the appropriate dye(s) or other markers are fed to reservoir 70 by line 72 and, in response to one or more signals from controller 64, feeds such dye(s) into injector 74 and thence into line 26, where the dye(s) mix with the fluid disposed within such line 26 and selectively label them.

After the labeled bodily fluid has been analyzed and, optionally, treated, and prior to the time it is returned via line 50 or 52 to either the body or to a reservoir, the marker/label (dye) may be removed from the fluid by conventional means. Thus, by way of illustration and not limitation, the label may be removed by means of an adsorption column 78 and/or by other adsorption means. Thus, e.g., the dye may be removed by other means, including chemical means. By way of illustration and not limitation, processes for stripping dyes or decolorizing various materials are known in the art. For example, U.S. Pat. No. 4,227,881 discloses a process for stripping dyes from textile fabric that includes heating an aqueous solution of an ammonium salt, a sulfite salt and an organic sulfonate to at least 140 degrees F. (60 degrees C.) and adding the dyed fabric to the heated solution while maintaining the temperature of the solution. U.S. Pat. No. 4,783,193 discloses a process for stripping color from synthetic polymer products by contacting the colored polymer with a chemical system.

It will be apparent that one can use one of several different physical and/or chemical means of removing the dye/marker/label from the bodily fluid; the aforementioned description is illustrative and not limitative. Regardless of which means are used, a purified bodily fluid is returned via line 50/52 to either the body or a reservoir.

During the purification process, additional material needed for such process may be charged via line 80, and/or dye and/or other waste material may be removed via line 80.

Referring again to Figure 4, the reservoir 70 may contain one or more labels/markers, and/or it may contain diluent to preferably dilute the bodily fluids so that preferably only one cell passes by any particular point in flow chamber 76 at anyone time. As will be apparent, this "laminar flow condition" facilitates the analyses of the bodily fluid by optical means.

Analysis

Referring again to Figure 1, in step 82 of the process the labeled bodily fluid is analyzed. One may conduct, e.g., flow cytometric analyses in accordance with the procedures described in the patents listed elsewhere in this specification; and one may use the devices disclosed in such patents for such analyses. Referring to Figure 4, the selectively marked bodily fluid(s) are then funneled into the flow chamber 76 of the particle analyzer 44, wherein they are subjected to analysis by conventional optical means.

One such analytical device is illustrated schematically in Figure 5. For the sake of simplicity of representation, unnecessary detail has been omitted from Figure 5. Referring to Figure 5, and in the embodiment depicted therein, a light source 84 is caused to focus on flow chamber 76. The amount of light transmitted through flow chamber 76 will vary with the properties of the bodily fluid within such chamber; see, e.g., United States patents 6,197,756, 6,197,593, 6,197,583, 6,197,582, 6,197,568, 6,197,540, and the like. The entire disclosure of each of these United States patents is hereby incorporated by reference into this specification.

Data Collection

Referring to Figure 5, the light transmitted through flow chamber 76 is detected by detector 86 which may, e.g., be a photodetector. Data is fed from detector 86 to controller 88.

Controller 88 is equipped with a database indicating the properties of normal bodily fluids. The property of any particular bodily fluid being analyzed can be compared with this database to determine whether they correlate. A lack of correlation may indicate a disease state, which can be thereafter treated by the particle analyzer 44.

Referring again to Figure 1, in step 90 data is collected from the analysis conducted in controller 88. Historical data may also be fed to the data collection device, either before, during, or after the analysis 82 of the bodily fluid. The collection of data in step 90, and its use, may be done in accordance with United States patent 6,197,593, the entire disclosure of which is hereby incorporated by reference into this specification.

Data from data collection step 90 may be added to from external sources. Alternatively, data from data collection step 90 may be exported to one or more external devices.

In one embodiment, not shown, when analysis step 82 and data collection step 90 indicate the presence of a dangerous abnormal condition within the bodily fluid, an external alarm is activated to warn the patient. When analysis 82 of the bodily fluid indicates that it is abnormal, the bodily fluid may be charged via line 92 to treatment step 94. As is indicated in Figure 5, treatments 110 may be conducted inline with analysis 108 within the flow chamber 76.

Treatment

Referring again to Figure 5, injector 96 is operatively connected to both detector 86 and controller 88 and, in response to signals there from, feeds energy and/or material to the bodily fluid to treat it.

One may feed radiation 98 to the bodily fluid to treat it. Thus, e.g., one may cause ultraviolet radiation to impact flow chamber 76 and to kill cancerous cell(s) disposed within such flow chamber 76. Thus, e.g., one may use electrical discharge 100 by means such as, e.g., electroporation. Thus, e.g., one may use magnetic fields 102. Thus, e.g., one may use sound particles and rays 104. Alternatively, or additionally, one may feed material via line 106 into flow chamber 76 that is adapted to kill or modify the abnormal cell(s).

One may use any of the materials commonly used to kill or modify cells. Thus, by way of illustration and not limitation, one may use gene vectors, viral particles, antibodies, chemotherapeutic agents, etc. Thus, e.g., one may do selective gene therapy on any particular cell.

To the extent, if any, there is a need to replenish material within injector 96, such material may be fed to injector 96 via line 115 from reservoir 116.

When it is desired to cause a particular cell to remain at a particular location for any period of time, the controller 64 can cause the closure of valves 112 and 114 so that fluid disposed between such valves cannot flow.

Because the particle analyzer 44 is capable of detecting one cell at a time, any abnormal cell detected at point 108 may be treated at point 110, e.g., the controller 88 determining precisely where such particular cell is at any point in time.

Return

As is illustrated in Figure 1, and in step 122, the cells or bodily fluid treated in step 94 may be returned to the body in step 122. In the preferred embodiment depicted in Figure 6, body

fluids that have been analyzed by particle analyzer 44 may be fed via line 50 to vessel 41, which may be the same or different from the blood vessel 40, from which the bodily fluid was sampled. Alternatively, or additionally, such analyzed bodily fluids may be fed via line 52 to reservoir 54 that, in the embodiment depicted, is disposed in a blood vessel 56.

Sorting

Referring again to Figure 1, the cells analyzed in step 82 may be sorted in sorting step 118 according to criteria detected by the detector 86 and analyzed by controller 88. In this sorting step, one may selectively segregate and collect certain cells within the bodily fluid. One may use conventional particle analyzer sorters in this step; see, e.g., United States patents 5,985,216 and 5,998,212, the entire disclosure of each of which is hereby incorporated by reference into this specification.

In one embodiment, stem cells are sorted from the bodily fluid. The identification and separation of such stem cells may be conducted by conventional means such as, e.g., the means disclosed in United States patent 5,665,557, the entire disclosure of which is hereby incorporated by reference into this specification.

The stem cells sorted in step 118 may be collected and thereafter used for many different purposes.

Maintenance

Figure 7 is a schematic of a means for maintaining bodily fluid (and/or a portion thereof) in maintenance step 120. Referring to Figure 7, some or all of the cells that have been sorted in sorting step 118 may be passed via line 52 to reservoir 54. In one embodiment, not shown, sorting step 118 is bypassed and bodily fluid is directly passed into reservoir 54.

In the embodiment depicted in Figure 7, reservoir 54 is disposed within blood vessel 56, and which is composed of porous material. In another embodiment, not shown, reservoir 54 may be disposed adjacent to a blood vessel, and/or be disposed adjacent to the intestines. This allows all necessary nutrients and supplies to be available to the retained cells. It also allows for waste products to be removed from reservoir 54. The porous material has a pore size that allows cells to remain within reservoir 54, but which allows nutrients and waste products to diffuse freely.

Removal

Referring to Figure 1, cells may be removed from the maintenance chamber, in removal step 124. Referring to Figure 7, one may remove some or all of the sorted material in step 118

and maintained in reservoir 54 by means, e.g., of syringe 60 and line 61. One may also withdraw fluid from reservoir 54 into blood vessel 56 by means of line 58.

Other Details

Location

The particle analyzer 44 may be disposed either within or without the patient's body-.Referring to Figure 8, it will be seen that a particle analyzer 44 is disposed in a patient's body. In the embodiment depicted in Figure 8, the particle analyzer 44 is disposed beneath a patient's skin, in the abdominal cavity. The particle analyzer 44 may be implanted within the patient's body by conventional means. Thus, by way of illustration and not limitation, one may implant particle analyzer 44 by the method disclosed in United States patent 6,198,950, the entire disclosure of which is hereby incorporated by reference into this specification. In the process of such patent, the implantable device is implanted under the skin in such a manner that the cannula projects into a blood vessel.

Thus, by way of further illustration, one may use the implantation processes and/or techniques disclosed in United States patents 6,198,969, 6,198,971, 6,198,965, 6,198,952, and the like. The entire disclosure of each of these United States patents also is incorporated by reference into this specification.

In another embodiment, illustrated in Figure 9, particle analyzer 44 is disposed outside the body 14 rather than inside it. In this embodiment, analyzer 44 may be removably attached to the body 14 by conventional means such as, e.g., belt 48 extending around the torso of the patient. The bodily fluid is sampled from, returned to or maintained in the body via cannulae tubes 26, 38, 50 or 52.

The particle analyzer 44 preferably has a weight of less than 12 pounds and, more preferably, weighs less than about 6 pounds. In one embodiment, the particle analyzer 44 is made from miniaturized components and weighs less than about 3 pounds. Technologies that enable this size and weight to be achieved include low energy lasers and advanced flow chambers that allow cells to flow in a narrowly focused laminar flow stream. Reference may be had, e.g., to United States patents 5,995,860 (implantable sensor for control of blood constituent levels), 6,057,149 (microscale devices adapted to move and mix microdroplets through microchannels), 6,119,031 (miniature spectrometer), 6,152,889 (body fluid sampler), 6,198,950 (implantable measurement device), and the like; the disclosure of each of these United States patents is hereby incorporated by reference into this specification.

Referring to Figure 5, the controller 64 is operatively connected to a power source 66.

In one embodiment, depicted in Figure 4, the mechanical action of pump 18 generates the power stored as power source 66. Thus, every output cycle of pump 18 provides some hydraulic pressure via line 68 to pump 66. This hydraulic pressure is converted into electrical power by conventional means such as, e.g., piezoelectric means.

In another embodiment, power source 66 is a battery. The battery may be rechargeable. Thus, in one aspect of this embodiment, the battery is recharged by electromagnetic radiation. The electromagnetic radiation may be transferred from a source disposed within the patient's body; or it may be transferred from a source external to the patient's body. Thus, e.g., a magnetic field may be produced by passing alternating current through a wire or coil, and this alternating magnetic field may be transmitted through a patient's skin into his body and coupled with a transducer, which produces alternating current from the alternating magnetic field.

In another embodiment, not shown, material and/or energy is fed to power source 66 via a line (not shown), and this material and/or energy is adapted to furnish power to power source 66. Thus, e.g., the material charged to power source 66 may undergo and/or facilitate a reaction, which produces energy consumed by power source 66.

Controllers

All controllers, including controller 64, controller 88 and any others used in other embodiments are able to be programmed with externally-generated signals 65, as diagrammed in Figures 4 and 5. All controllers are additionally able to interface with and import data from external databases (not shown).

Materials

Referring to Figure 3, in one embodiment, the casing 22, of pump 18 is made from a flexible, elastic biocompatible material. In embodiments where the particle analyzer is located subcutaneously, the particle analyzer is made from biocompatible materials such as surgical steel or encased in biocompatible materials. All cannulae and tube are made from flexible, biocompatible materials. Flow chamber 76 is preferably transparent to the desired light source.

Bodily Fluids

In the preferred embodiment depicted in all figures, particle analyzer 44 is sampling blood. In another embodiment, not shown, the particle analyzer 44 is so disposed that it samples bodily liquids such as, e.g., lymph, bone marrow, spinal fluid, and the like. As will be apparent to those skilled in the art, the particle analyzer 44 is adapted to sample and analyze and treat unmodified bodily liquids, that is, bodily liquids occurring in their natural state within the body.

It is to be understood that the aforementioned description is illustrative only and that changes can be made in the apparatus, in the ingredients and their proportions, and in the sequence of combinations and process steps, as well as in other aspects of the invention discussed herein, without departing from the scope of the invention as defined in the following claims.

We claim:

1. A particle analyzer comprising means for acquiring particles of cellular material from a living body, means for labeling said particles of cellular material from said living body to produce labeled particles, means for analyzing said labeled particles to produce analyzed and labeled particles, means for sorting said analyzed and labeled particles to produce labeled and sorted particles, and means for maintaining a portion of said analyzed and labeled particles in a viable state to produce labeled and viable particles.

2. The particle analyzer as recited in claim 1, further comprising means for modifying a portion of said analyzed and labeled particles, thereby producing modified and labeled particles.

3. The particle analyzer as recited in claim 2, further comprising means for returning said modified and labeled particles to said living body.

4. The particle analyzer as recited in claim 3, wherein said bodily fluid is blood.

5. The particle analyzer as recited in claim 5, wherein said means for labeling said particles of cellular material is a dye.

6. The particle analyzer as recited in claim 5, wherein said dye is a cell membrane permeant, red-excited dye.

7. The particle analyzer as recited in claim 6, wherein said means of maintaining a portion of said analyzed and labeled particles in a viable state comprises a reservoir.

8. The particle analyzer as recited in claim 7, wherein said reservoir is comprised of a permeable membrane.

9. The particle analyzer as recited in claim 8, further comprising means for removing said a portion of said analyzed and labeled particles from said reservoir.

10. A particle analyzer comprising means for acquiring particles of cellular material from a living body, means for labeling said particles of cellular material from a living body to produce labeled particles, means for analyzing said labeled particles to produce analyzed and labeled particles, means for sorting said analyzed and labeled particles to produce labeled and sorted particles, and means for modifying a first portion of said analyzed and labeled particles to produce labeled and modified particles.

11. The particle analyzer as recited in claim 10, further comprising means for maintaining a portion of said analyzed and labeled particles in a viable state to produce labeled and viable particles.

12. The particle analyzer as recited in claim 10, further comprising means for returning said modified and labeled particles to said living body.

13. The particle analyzer as recited in claim 11, further comprising means for returning said modified and labeled particles to said living body.

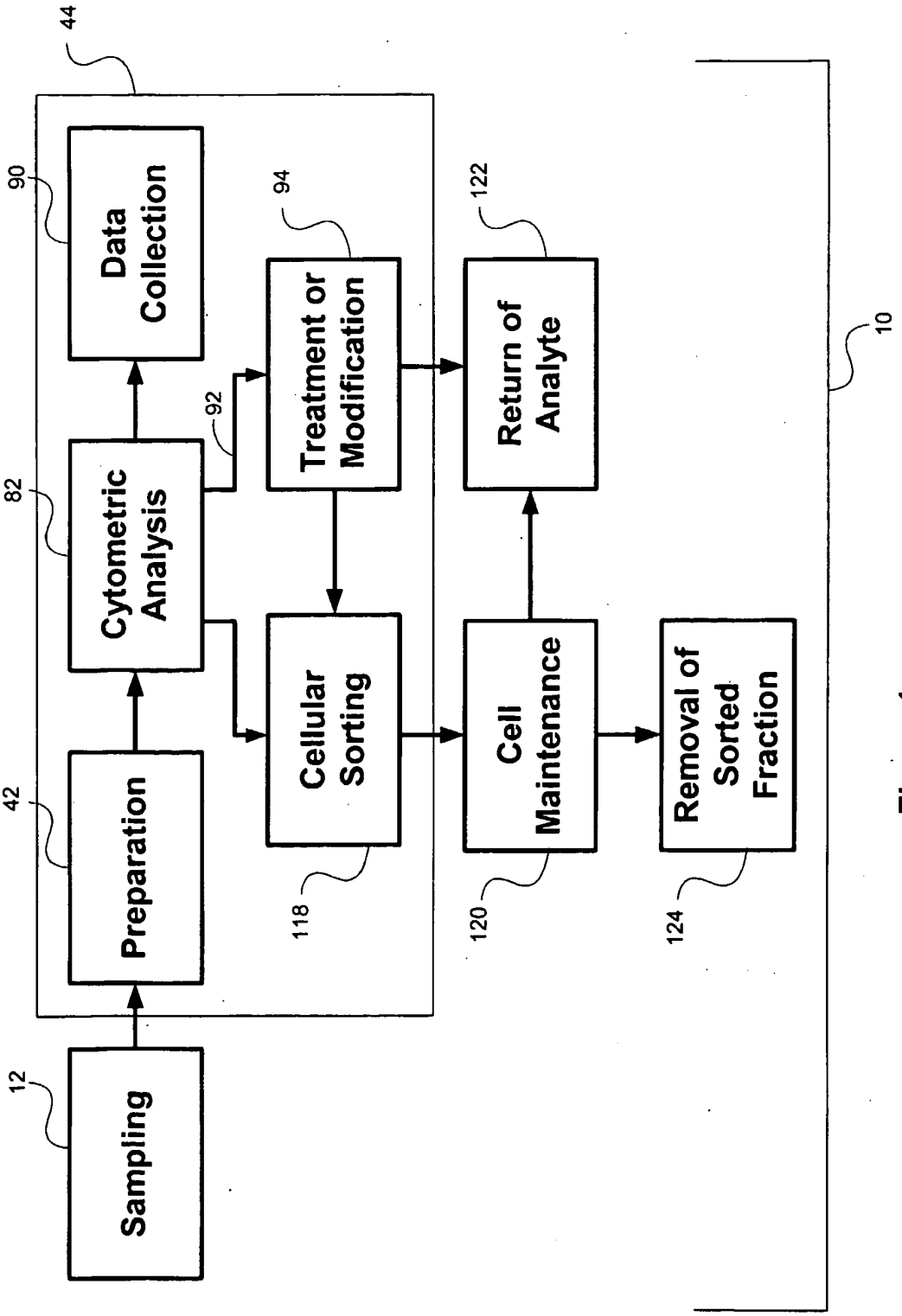
14. The particle analyzer as recited in claim 1, further comprising a pump hydraulically connected to said particle analyzer.

15. The particle analyzer as recited in claim 14, wherein said pump is disposed within said living body.

16. The particle analyzer as recited in claim 15, further comprising means for producing electrical power upon the actuation of said pump.

17. The particle analyzer as recited in claim 1, wherein said particle analyzer is disposed within said living body.

18. The particle analyzer as recited in claim 1, wherein said particle analyzer weighs less than about 3 pounds.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Preparation
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Cytometric Analysis
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Data Collection
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Treatment or Modification
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Cellular Sorting
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Cell Maintenance
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Removal of Sorted Fraction
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Figure 1

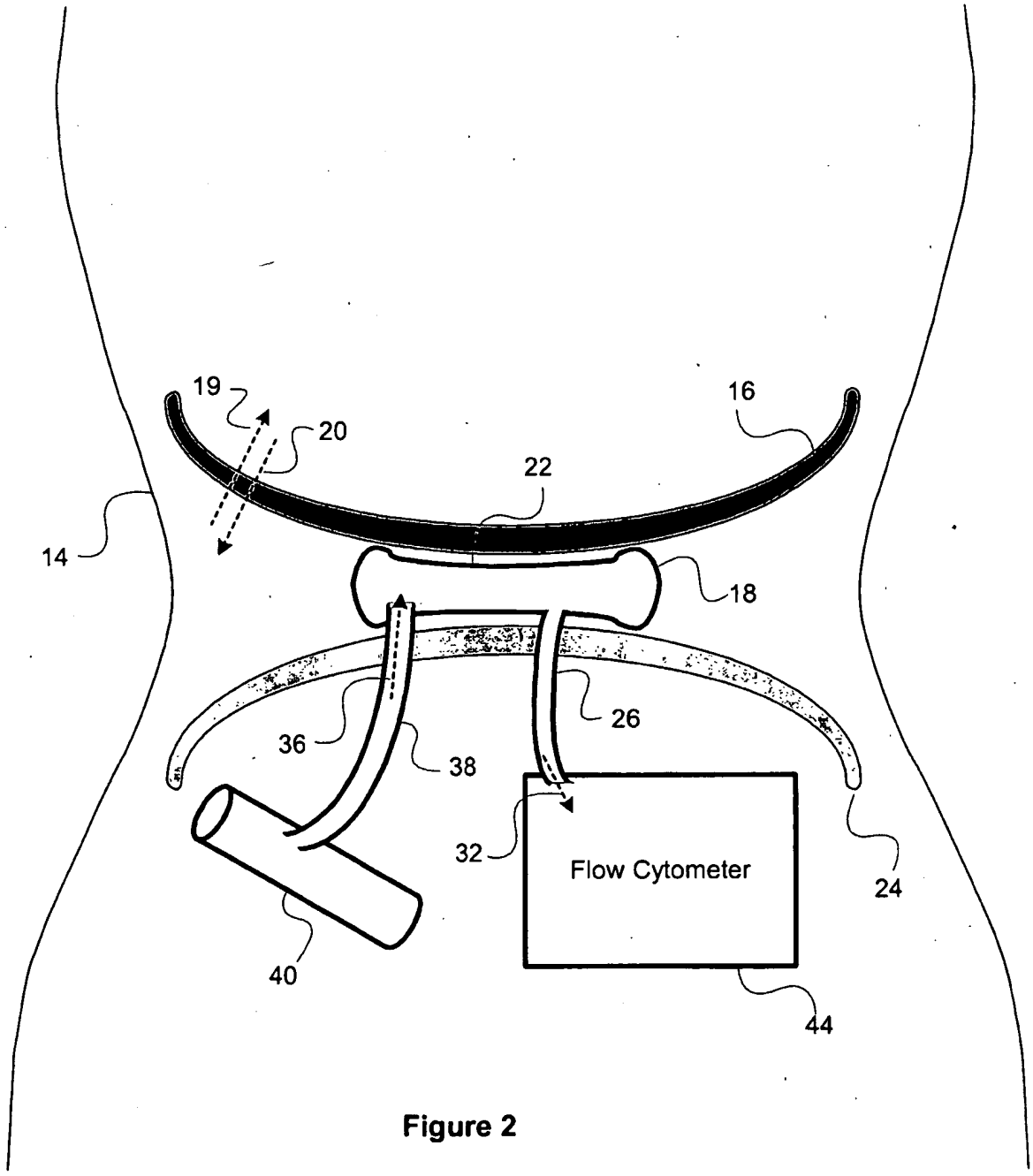


**Figure 2**

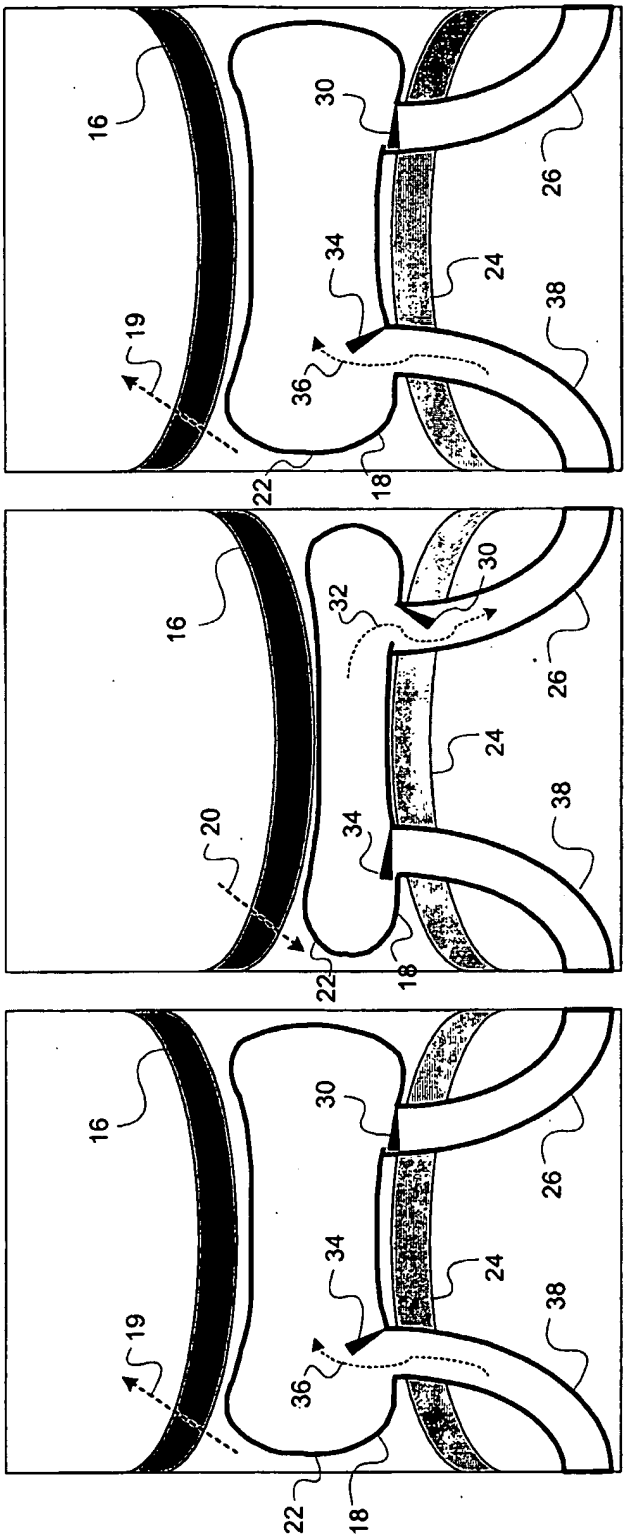
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Figure 3

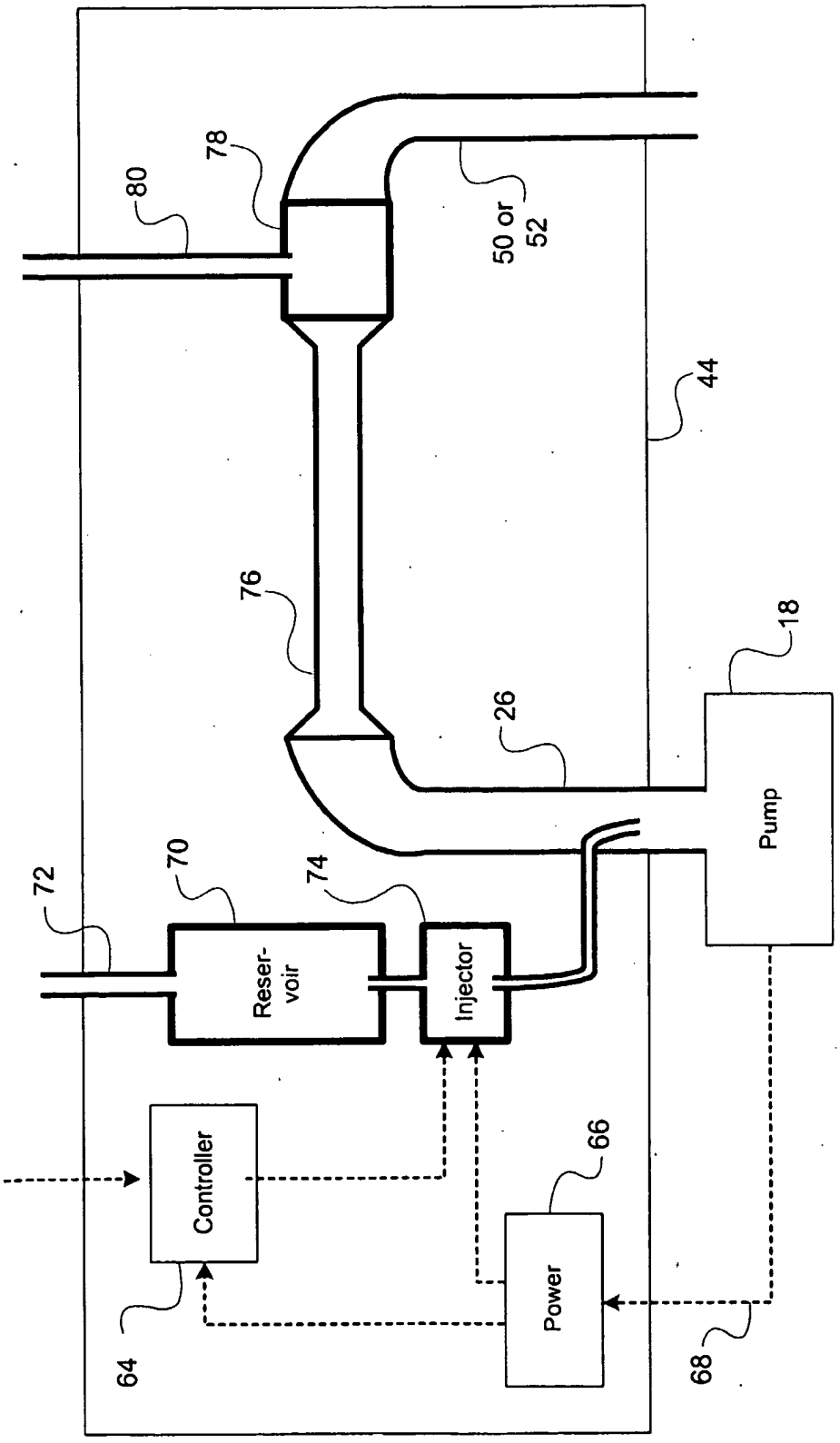
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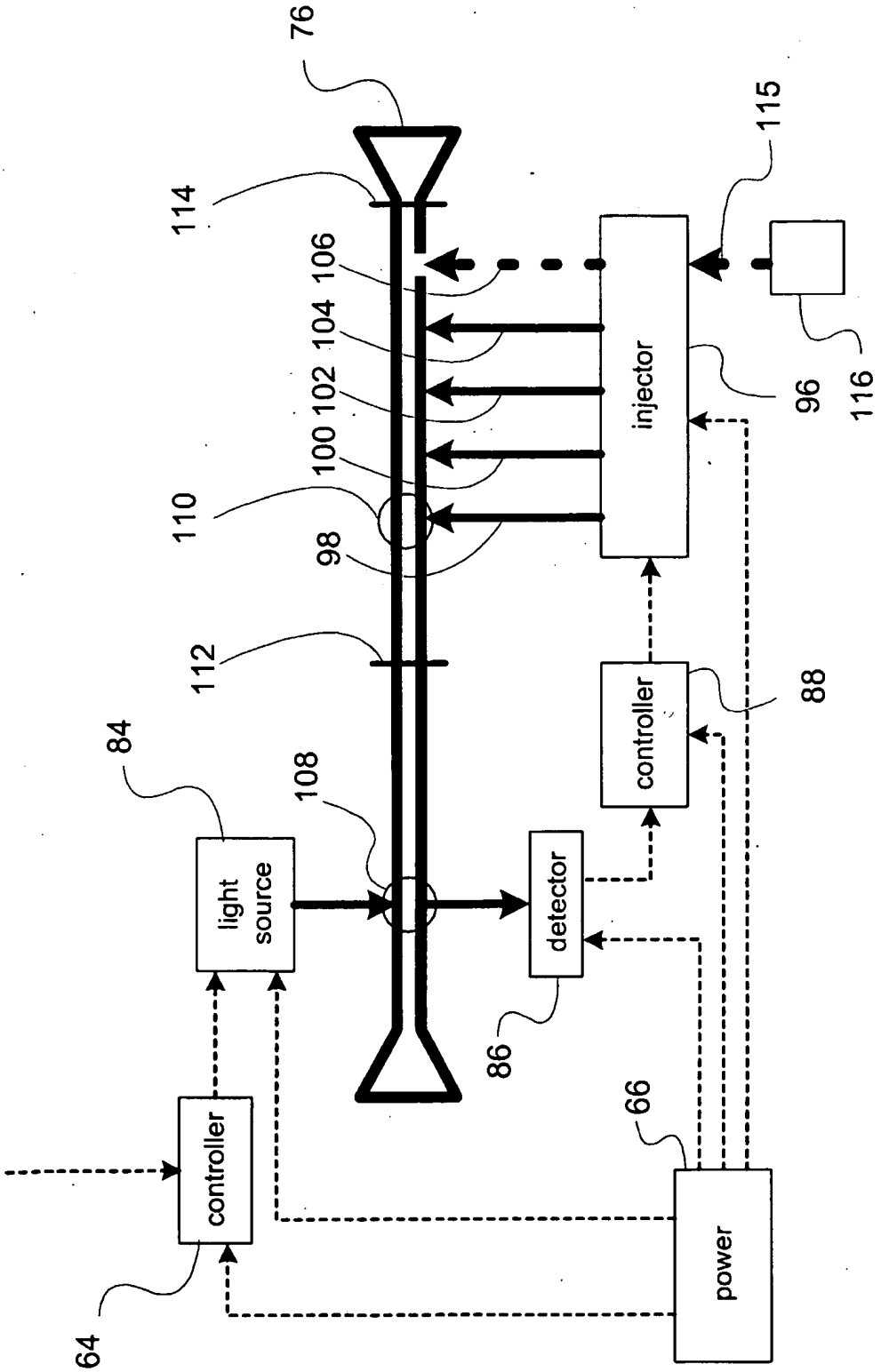
Figure 4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86
66
power
controller
64

Figure 5

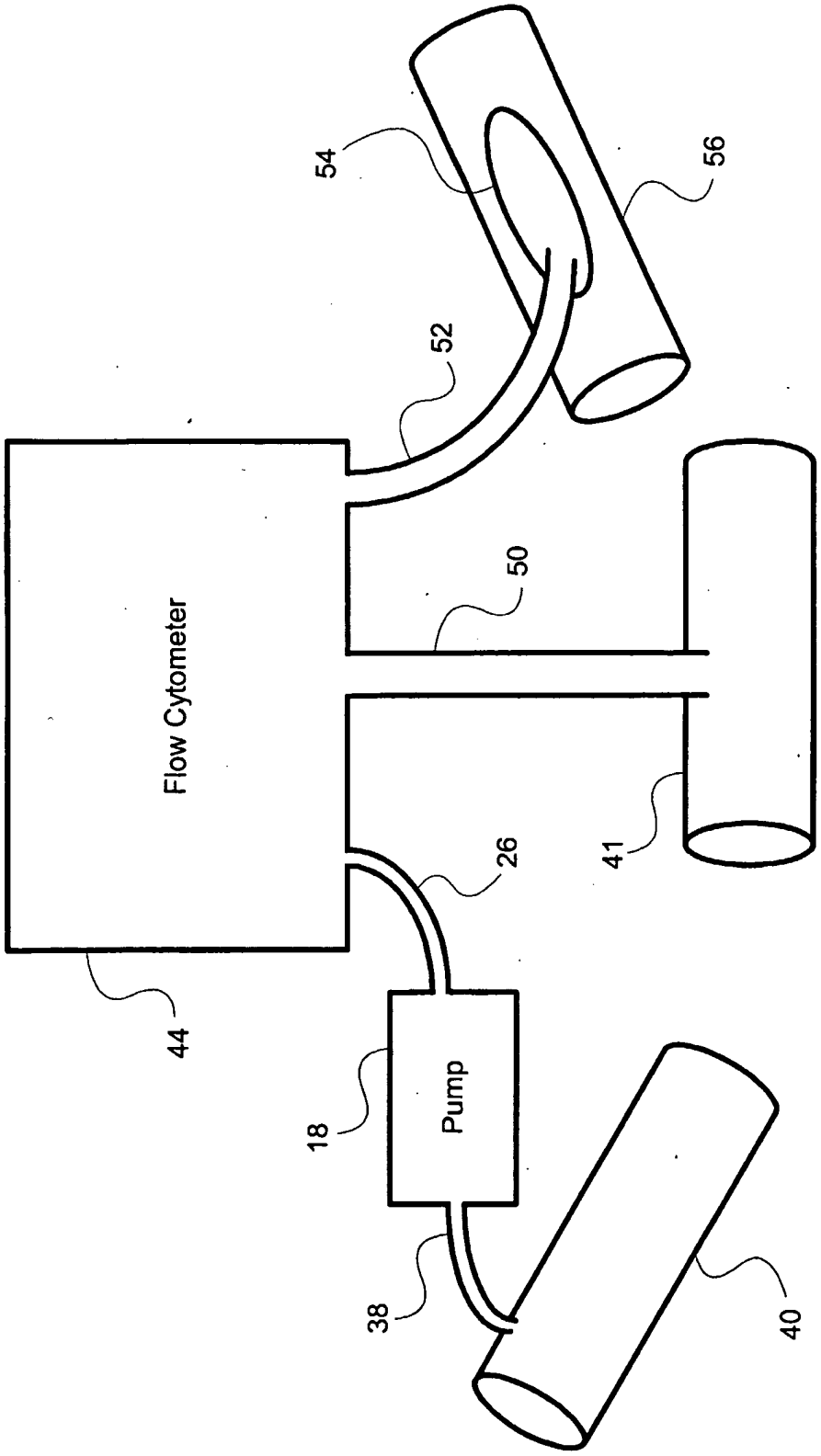


Figure 6

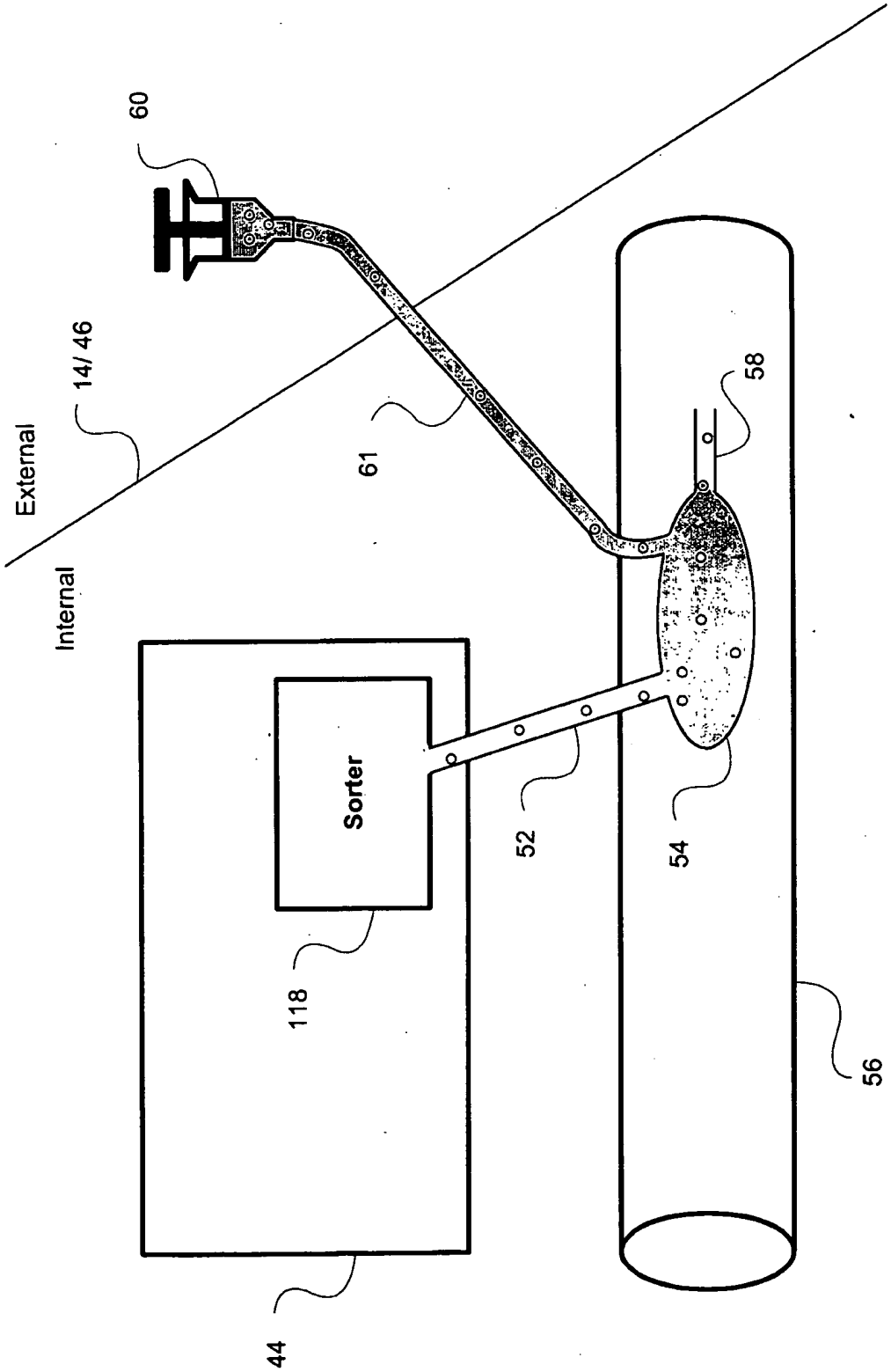


Figure 7

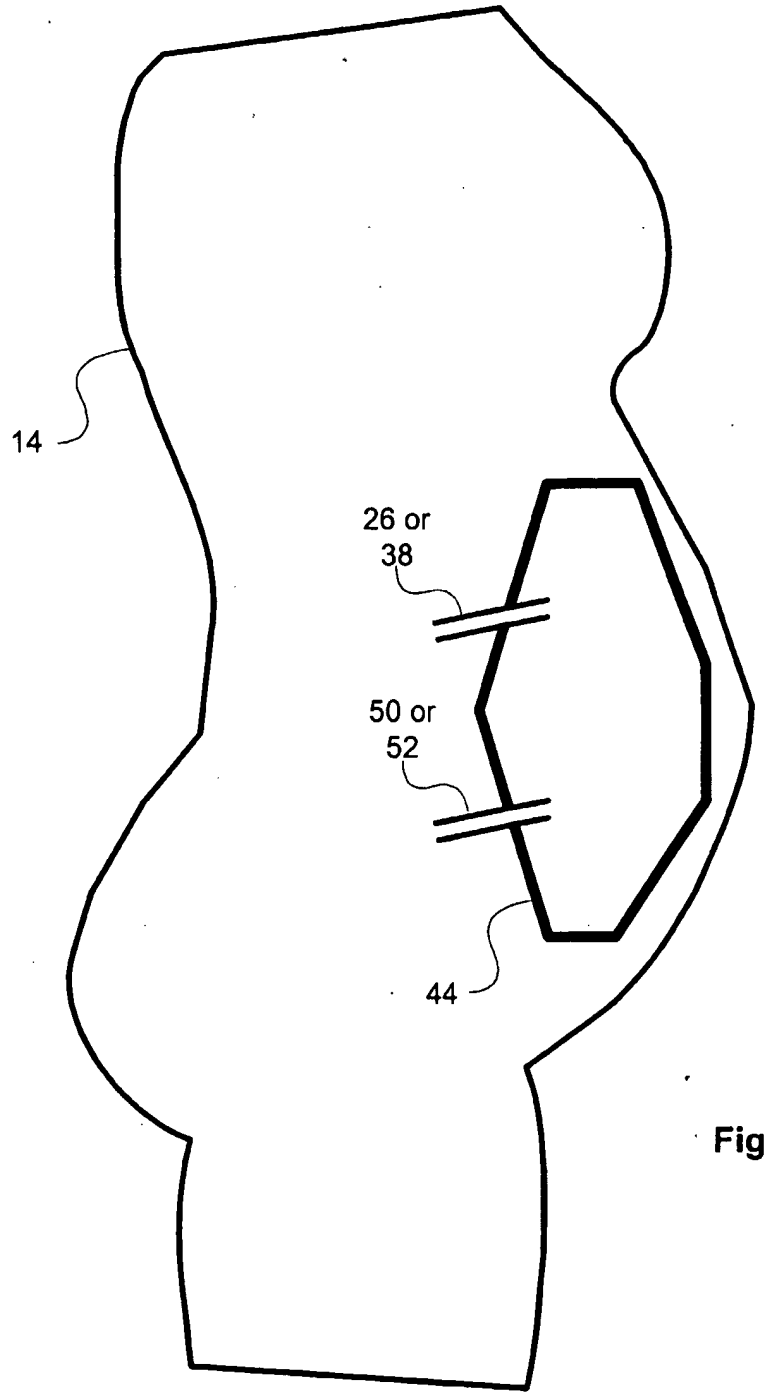


Figure 8

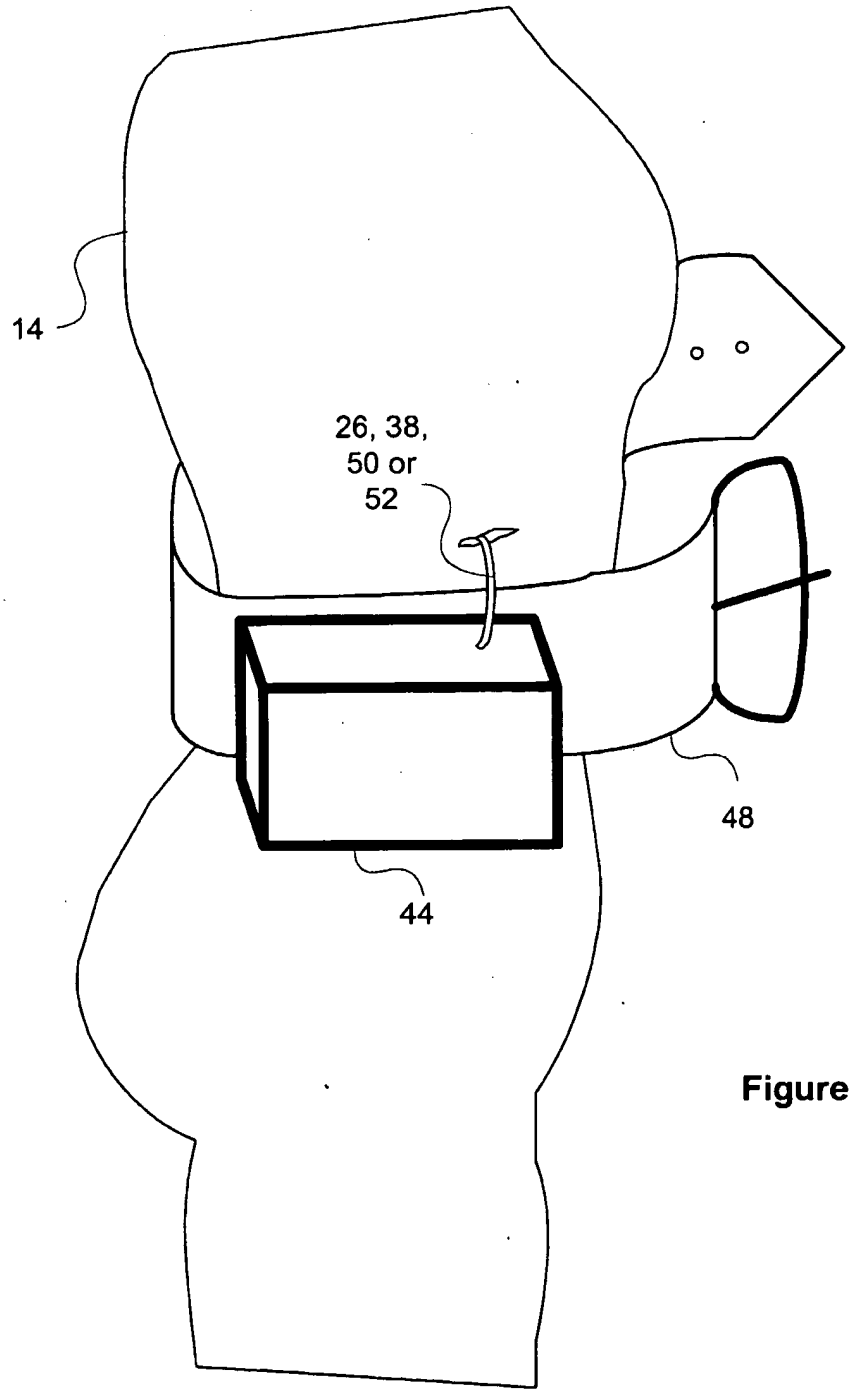


Figure 9

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/14253

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61M 37/00; G01N 33/48

US CL : 604/4.01; 436/63

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 604/4.01; 436/63

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST BRS (flow cytometer, particle analyzer, blood)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 5,057,413 A (TERSTAPPEN et al) 15 October 1991 (15.10.1991), entire document. | 1-18 |
| Y | US 5,374,401 A (von BERG) 20 December 1994 (20.12.1994), column s 3-4. | 1,3,4,7,9-11,14,15,18 |
| Y | US 5,444,527 A (KOSAKA) 22 August 1995 (22.08.1995), entire document. | 1,10 |
| A | US 5,703,959 A (ASANO et al) 30 December 1997 (30.12.1997), entire document | 1-18 |
| Y | US 5,907,240 A (CARVER, Jr., et al) 25 May 1999 (25.05.1999), columns 4-6. | 1-18 |
| Y | US 6,097,485 A (LIEVAN) 01 August 2000 (01.08.2000), entire document. | 10,14-18 |
| Y | US 6,193,891 B1 (KENT et al) 27 February 2001 (27.02.2001), entire document. | 2-6 |
| Y | US 6,197,593 B1 (DEKA et al) 06 March 2001 (06.03.2001), entire document. | 1,4-8 |
| Y,P | US 6,281,018 B1 (KIROUAC et al) 28 August 2001 (28.08.2001), entire document. | 1-9 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 15 July 2002 (15.07.2002) | 10 SEP 2002 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703)305-3230 | Authorized officer<br>Leslie Deak<br>Telephone No. 703-305-0200 |

Form PCT/ISA/210 (second sheet) (July 1998)